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Research Note

Differentiation of *Cylicocyclus gyalocephaloides* of *Equus burchelli* from *Cylicocyclus insigne* of *Equus caballus* (Strongyloidea: Nematoda)

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ABSTRACT: Specimens of *Cylicocyclus insigne* (Boulenger, 1917) from *Equus caballus* and *Cylicocyclus gyalocephaloides* Ortlepp, 1938 from *Equus burchelli* were studied to determine whether they should be recognized as separate species. Both are relatively large species with broader than deep buccal capsules with thick, concave walls and a distinct basal external ring; a large esophageal funnel without a thick cuticular lining; and an excretory pore and cervical papillae at or near the junction of the esophagus and intestine. Although the specimens of *C. gyalocephaloides* are as long or longer than *C. insigne*, the former have a longer esophagus, males have shorter spicules and a longer gubernaculum, and females have a shorter vagina. In addition, the dorsal papillae of the genital cone of *C. gyalocephaloides* are more slender than those of *C. insigne* and bilateral spines present on the genital cones of *C. insigne* were not found on the available specimens of *C. gyalocephaloides*. It was concluded that sufficient morphological differences are present between *C. insigne* of *E. caballus* and *C. gyalocephaloides* of *E. burchelli* to recognize and distinguish both nematode species.

KEY WORDS: Nematoda, Strongyloidea, Cyathostominea, cyathostomins, *Cylicocyclus insigne*, *Cylicocyclus gyalocephaloides*, *Equus caballus*, horse, *Equus burchelli*, zebra, donkey, systematics, nomenclature, Africa.

Among the 52 nematode species of the tribe Cyathostominea, strict host specificity for species of equids is not common (Lichtenfels et al., 1998). However, several species are known only from 1 species of host. The cosmopolitan species, *Cylicocyclus insigne* (Boulenger, 1917), has been reported (see checklist of Round [1968]) from horses, donkeys, and zebras in Africa. Others (Boulenger, 1920; Ortlepp, 1938; Krecek et al., 1987) recognized *C. insigne*–like nematodes from zebras as separate species. Boulenger (1917) described *C. insigne* from the horse, *Equus caballus*. He later (Boulenger, 1920) described *Cylicocyclus zebrae* Boulenger, 1920 from a ''zebra.'' Subsequently, Boulenger (1921) synonymized *C. zebrae* with *C. insigne*. Most workers followed Boulenger's (1921) synonomy (see Round [1968]) until the description of *Cylicocyclus gyalocephaloides* Ortlepp, 1938 again proposed that the *C. insigne*–like nematodes of the horse and zebras are separate species. Subsequently, *C. gyalocephaloides* has been reported only rarely. Krecek et al. (1987) reported *C. gyalocephaloides* from *Equus burchelli* (Burchell's zebra) from Kruger National Park in South Africa, and Oliveira et al. (1994) reported both *C. gyalocephaloides* and *C. insigne* from *Equus asinus* (donkey) from Brazil. Lichtenfels et al. (1998) placed *C. gyalocephaloides* on a list of species inquirendae and recommended that it be restudied to determine whether it could be differentiated from *C. insigne*.

As part of a revision of the systematics of the Cyathostominea of equids, we studied all available specimens, including types, of *C. gyalocephaloides* and compared them with specimens of *C. insigne*. We obtained 5 females and 2 males of the type series of *C. gyalocephaloides* from the National Collection of Animal Helminths, University of Pretoria, Onderstepoort, South Africa (formerly the Onderstepoort Helminthological Collection) (Collection number T.2099). We also studied 8 females from *E. burchelli* (United States National Parasite Collection [USNPC] 91995) reported by Krecek et al. (1987). In addition, we discovered in the USNPC (47008) 20 specimens, including 4 males, identified by J. T. Lucker as *C. insigne*, that were collected in 1948 by A. Loveridge in Nyasaland, Africa (Malawi) from *E. burchelli*. We found the above 2 lots from zebras to be conspecific with the type specimens of *C. gyalocephaloides*. We were unable to locate any records of the types of *C. insigne* or *C. zebrae* (Gibbons and Harris, personal

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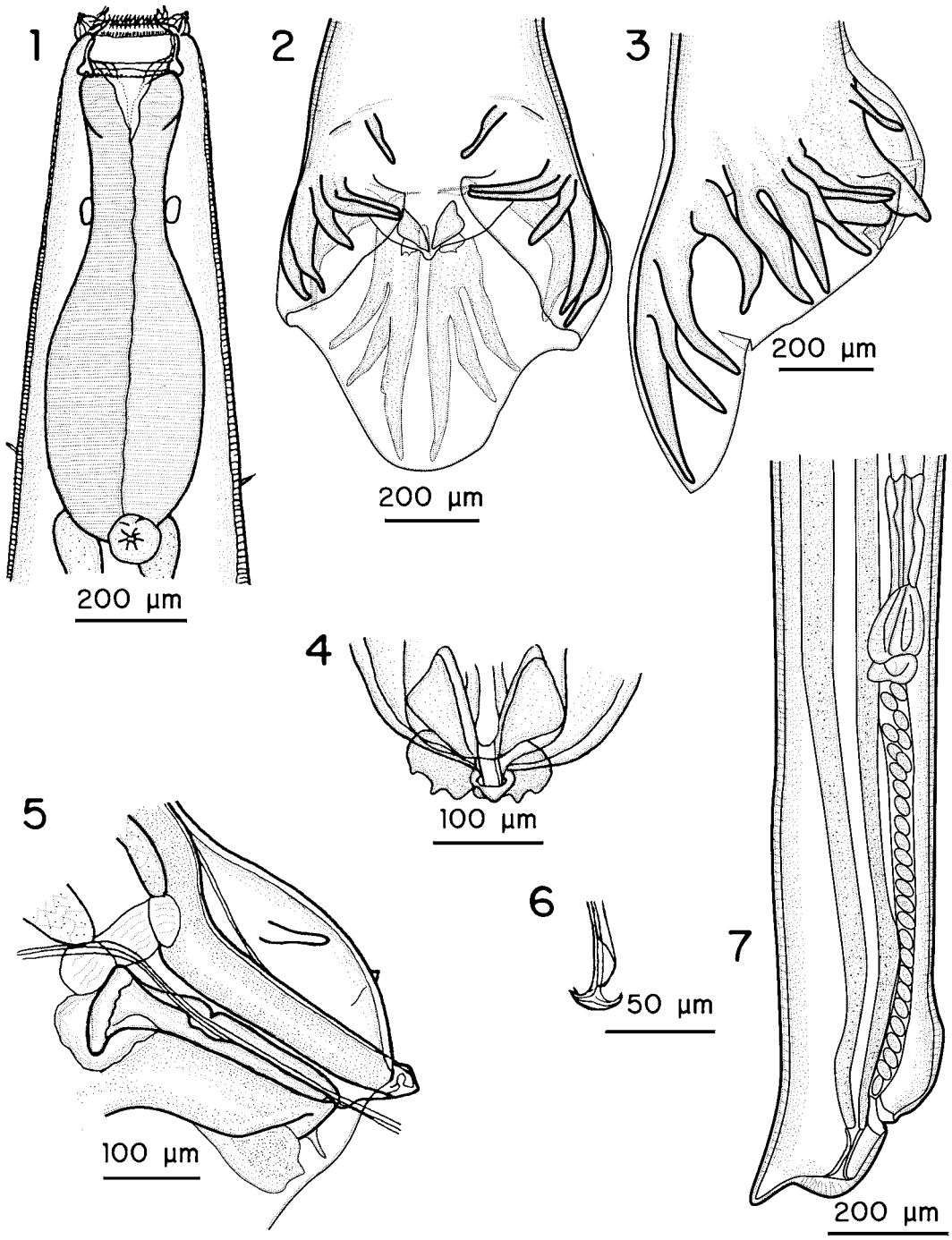
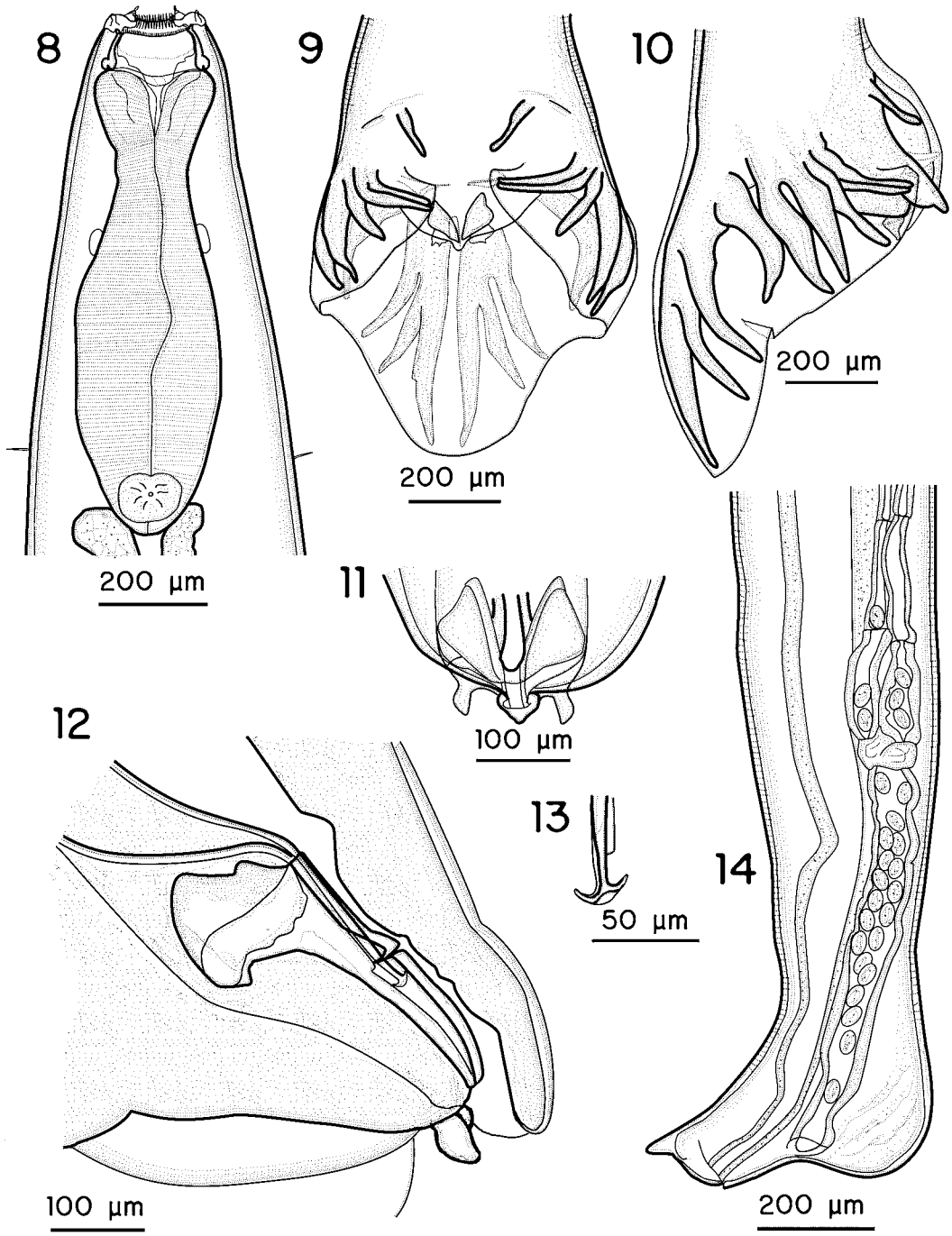


Figures 1–7. *Cylicocyclus insigne*. **1.** Esophageal region, ventral view, showing shape of esophagus and positions of ventral excretory pore and lateral cervical papillae at base of esophagus. **2.** Male tail, ventral view. **3.** Male tail, lateral view. **4.** Male genital cone, ventral view. **5.** Male genital cone and gubernaculum, lateral view. **6.** Distal ends of spicules, ventral view, showing pick-like tips on each spicule. **7.** Female tail, lateral view.



Figures 8–14. *Cylicocyclus gyalocephaloides*. **8.** Esophageal region, ventral view, showing shape of esophagus and positions of ventral excretory pore and lateral cervical papillae at base of esophagus. **9.** Male tail, ventral view. **10.** Male tail, lateral view. **11.** Male genital cone, ventral view. **12.** Male genital cone and gubernaculum, lateral view. **13.** Distal ends of spicules, ventral view, showing pick-like tips on each spicule. **14.** Female tail, lateral view.

Table 1. Comparative measurements*† of different lots of *Cylicocyclus gyalocephaloides* from zebras (probably all *Equus burchelli*) and *Cylicocylus insigne* from *Equus caballus*.

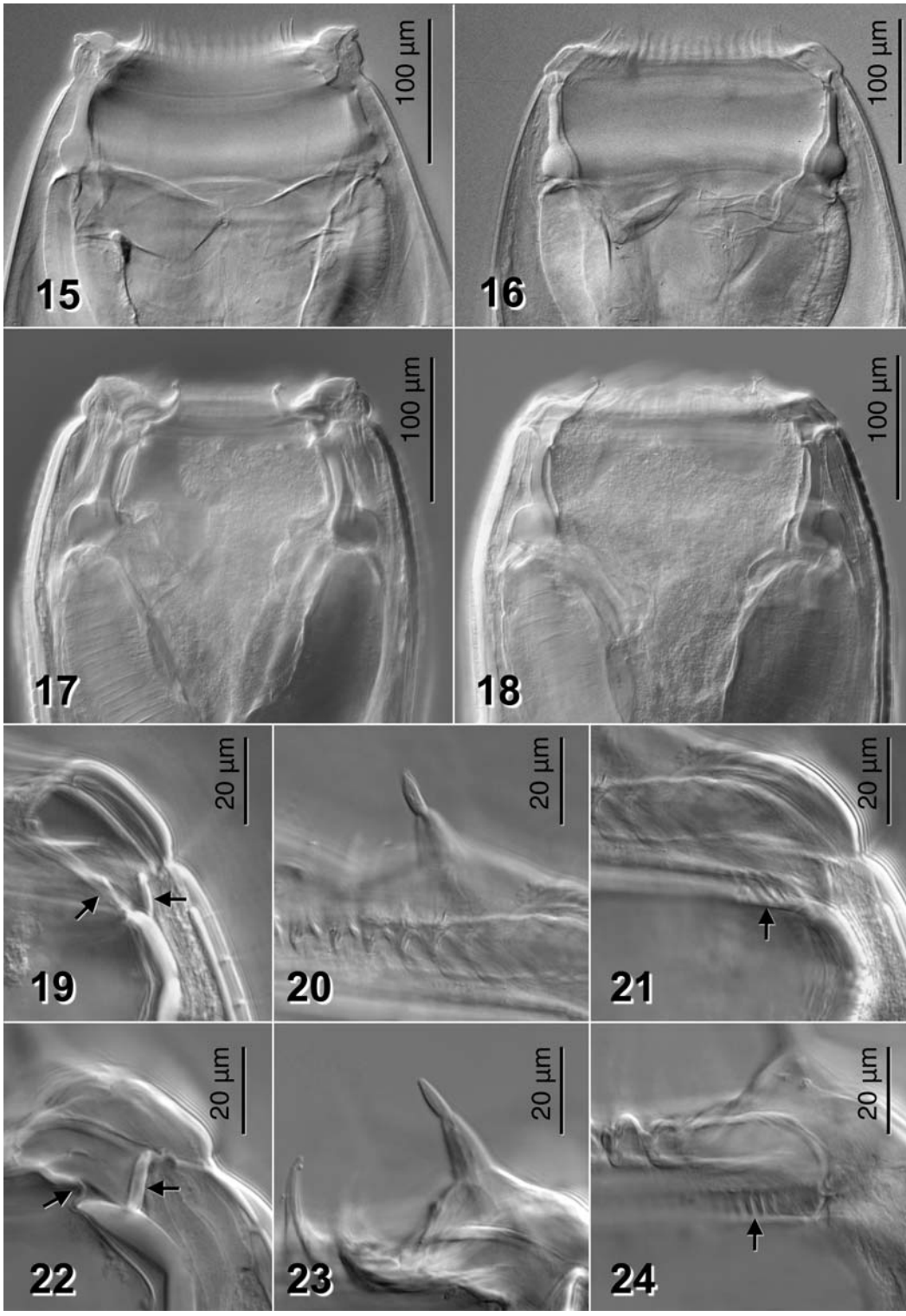
	Cylicocyclus gyalocephaloides					*Cylicocyclus insigne*	
	Type series		USNPC 91995	USNPC 47008		Institute of Zoology NAS of Ukraine	
Character	♂ (N = 2)	♀ (N = 5)	♀ (N = 8)	♂ (N = 5)	♀ (N = 9)	♂ (N = 30)	♀ (N = 31)
L Body (mm)	9.0	6.5–11.0 (8.9 ± 1.6)	12.5–16.0 (14.8 ± 1.1)	10.0–14.0 (12.7 ± 1.6)	11.0–16.0 (13.8 ± 1.8)	10.2–12.8 (11.6 ± 0.7)	10.4–15.2 (13.0 ± 1.0)
W Body	687–707 (697)	746–805 (770 ± 26)	687–805 (766 ± 38)	864–982 (907 ± 47)	982–1,080 (1,045 ± 36)	472–688 (593 ± 67)	406–936 (785 ± 114)
W buccal capsule	195–220 (207)	146–259 (208 ± 41)	195–244 (223 ± 19)	185–224 (210 ± 15)	205–254 (231 ± 15)	138–188 (163 ± 11)	175–264 (211 ± 28)
D buccal capsule	68	73–88 (80 ± 6)	68–102 (85 ± 11)	83–98 (91 ± 6)	93–107 (100 ± 4)	53–81 (66 ± 5)	65–85 (76 ± 5)
L esophagus	820–869 (844)	888–1,015 (955 ± 47)	956–1,044 (994 ± 29)	962–1,060 (1,013 ± 47)	1,060–1,198 (1,134 ± 52)	525–872 (819 ± 73)	861–1,112 (985 ± 56)
W esophagus	342	244–342 (305 ± 39)	293–342 (322 ± 16)	317–342 (336 ± 11)	293–386 (352 ± 26)		
Cervical papillae to anterior end		878–976 (913 ± 46)	878–1,074 (1,015 ± 62)	917–1,005 (968 ± 33)	942–1,237 (1,085 ± 83)	558–888 (731 ± 60)	760–960 (833 ± 58)
Excretory pore to anterior end	878–898 (888)	927–1,044 (960 ± 49)	996–1,220 (1,126 ± 82)	996–1,074 (1,038 ± 34)	1,080–1,335 (1,198 ± 82)	600–896 (814 ± 57)	784–1,138 (964 ± 75)
Nerve ring to anterior end	293–322 (307)	342–439 (377 ± 41)	410–498 (458 ± 29)	429–478 (461 ± 19)	432–530 (478 ± 37)	308–416 (365 ± 23)	384–472 (442 ± 21)
L dorsal ray	586			785–1,001 (923 ± 81)		598–708 (663 ± 33)	
L spicules (mm)	2.1			2.2–2.6 (2.4 ± 0.2)		2.6–3.4 (3.0 ± 0.2)	
L gubernaculum	293–307 (300)			342–371 (359 ± 11)		248–322 (287 ± 16)	
L vagina		439–976 (709 ± 218)	683–927 (781 ± 82)		429–1,074 (673 ± 195)		747–2,136 (1,403 ± 393)
Vulva to tip of tail		283–415 (327 ± 52)	327–464 (382 ± 49)		342–537 (423 ± 66)		329–512 (426 ± 54)
L tail		122–220 (161 ± 37)	122–195 (154 ± 27)		176–244 (221 ± 29)		163–271 (223 ± 33)
L egg		92–98 (93 ± 3)	79–98 (90 ± 5)		92–110 (100 ± 7)		73–96 (85 ± 6)
W egg		49–61 (55 ± 4)	49–61 (53 ± 6)		55–61 (60 ± 2)		41–53 (45 ± 3)
L vestibulum		78–137 (105 ± 22)	88–146 (101 ± 19)		98–107 (100 ± 5)		62–105 (86 ± 9)
L sphincter		205–293 (244 ± 33)	283–371 (338 ± 27)		322–439 (372 ± 37)		171–304 (236 ± 41)
L infundibulum		176–273 (221 ± 39)	244–371 (304 ± 38)		293–390 (337 ± 38)		208–396 (294 ± 41)

* D, depth; L, length; W, width.

† Measurements are presented as range values followed parenthetically by mean ± SE. All measurements are presented in micrometers unless otherwise noted.

communication). Specimens of *C. insigne* that we studied included specimens of 4 lots from *E. caballus* from Kazakhstan (1 lot, USNPC 93167; 3 lots, G. M. Dvojnos Collection, National Academy of Sciences of Ukraine, Kiev, Ukraine) and 2 lots from *E. caballus* from Philippines and United States (USNPC 50862 and 73046). We attempted to include specimens of *C. insigne* from other hosts from Africa, but all available lots were redetermined by us as other species. Three lots of specimens identified as *C. zebrae* collected from *E. burchelli* in Africa were borrowed from the Natural History Museum, London, U.K., but were found to be other species.

Our comparative studies used interference-contrast light microscopy of whole specimens cleared in phenol–alcohol (80% melted phenol crystals in 20%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absolute ethanol). Drawings (Figs. 1–14) were prepared with the aid of a camera lucida. Photomicrographs were made with a digital camera, and the halftone plates (Figs. 15–28) were prepared using Adobe Photoshop. All measurements are given in microns unless otherwise indicated.

The results of our comparisons of type specimens of *C. gyalocephaloides* and additional specimens from zebras with specimens of *C. insigne* from *E. caballus* indicate that the specimens from the 2 hosts are very similar and share many characteristics (Figs. 1–28; Table 1). Both are relatively large species with broader than deep buccal capsules with thick, concave walls and a distinct basal external ring; a large esophageal funnel without a thick cuticular lining; and an excretory pore and cervical papillae at or near the junction of the esophagus and intestine. The specimens of *C. gyalocephaloides* are as long or longer than *C. insigne*, but the former have a longer esophagus, males have shorter spicules and a longer gubernaculum, and females have a shorter vagina (Table 1). In addition, the dorsal papillae of the genital cones of *C. gyalocephaloides* (Figs. 11, 12, 27, 28) are more slender than those of *C. insigne* (Figs. 4, 5, 25, 26), and bilateral spines present on the genital cones of *C. insigne* (Figs. 5, 26) were not found on the available specimens of *C. gyalocephaloides*.

The differences between the lots from zebras and those from horses suggest that they are different species. Although it is possible that differences in measurements of nematodes can result from being in a different host, such host effects often result in concordant reductions in body lengths and reduced development of reproductive organs (Knight, 1968). Conversely, using experimental infections, Lichtenfels (1971) showed that a nematode in an unusual host may have increased body lengths; but, in both stunted and larger specimens resulting from short-term host change, spicule size paralleled body size. In the case of *C. gyalocephaloides* in zebras, the specimens are clearly more robust in size, but spicules and vaginas are shorter, and the gubernaculum is longer.

On a practical basis, distinguishing between these 2 similar species can be done initially on the basis of host species. There is little evidence that *C. gyalocephaloides* is commonly found anywhere but in zebras (probably all *E. burchelli*) in Africa (but see Oliveira et al. [1994]), and we suspect that records of *C. insigne* in zebras may be *C. gyalocephaloides* instead. When a large species of *Cylicocyclus* is found in zebras, with characteristics similar to *C. insigne* (large, thinly lined esophageal funnel with excretory pore and cervical papillae near the junction of the esophagus and intestine), the spicule and vagina lengths can be used to distinguish between *C. gyalocephaloides* and *C. insigne*.

Other large species of *Cylicocyclus* reported from Africa include: *Cylicocyclus elongatus elongatus* (Looss, 1900), which can be distinguished by a thickly lined esophageal funnel, a cylindrical rather than club-shaped esophagus, and excretory pore and cervical papillae near the nerve ring; *Cylicocyclus auriculatus* (Looss, 1900), which can be distinguished by its exceptionally long lateral cephalic papillae (amphids), its unique submedian papillae with exceptionally long, thin tips, and a small esophageal funnel with a thick cuticular lining; and *Cylicocyclus adersi* (Boulenger, 1920), which can be distinguished by its much smaller ring at the base of the buccal capsule, a relatively small esophageal funnel with a thick cuticular lining, and a short but distinct dorsal gutter (Kharchenko et al., 2004).

Most of the large species of *Cylicocyclus* discussed in this study are common parasites of *E. asinus* or *E. burchelli* in Africa; exceptions being *C. insigne* and *Cylicocyclus elongatus kotlani* Ihle, 1920. It is tempting to speculate that this group of similar species originated in Africa in donkeys and zebras, with *C. insigne* and *C. elongatus kotlani* colonizing

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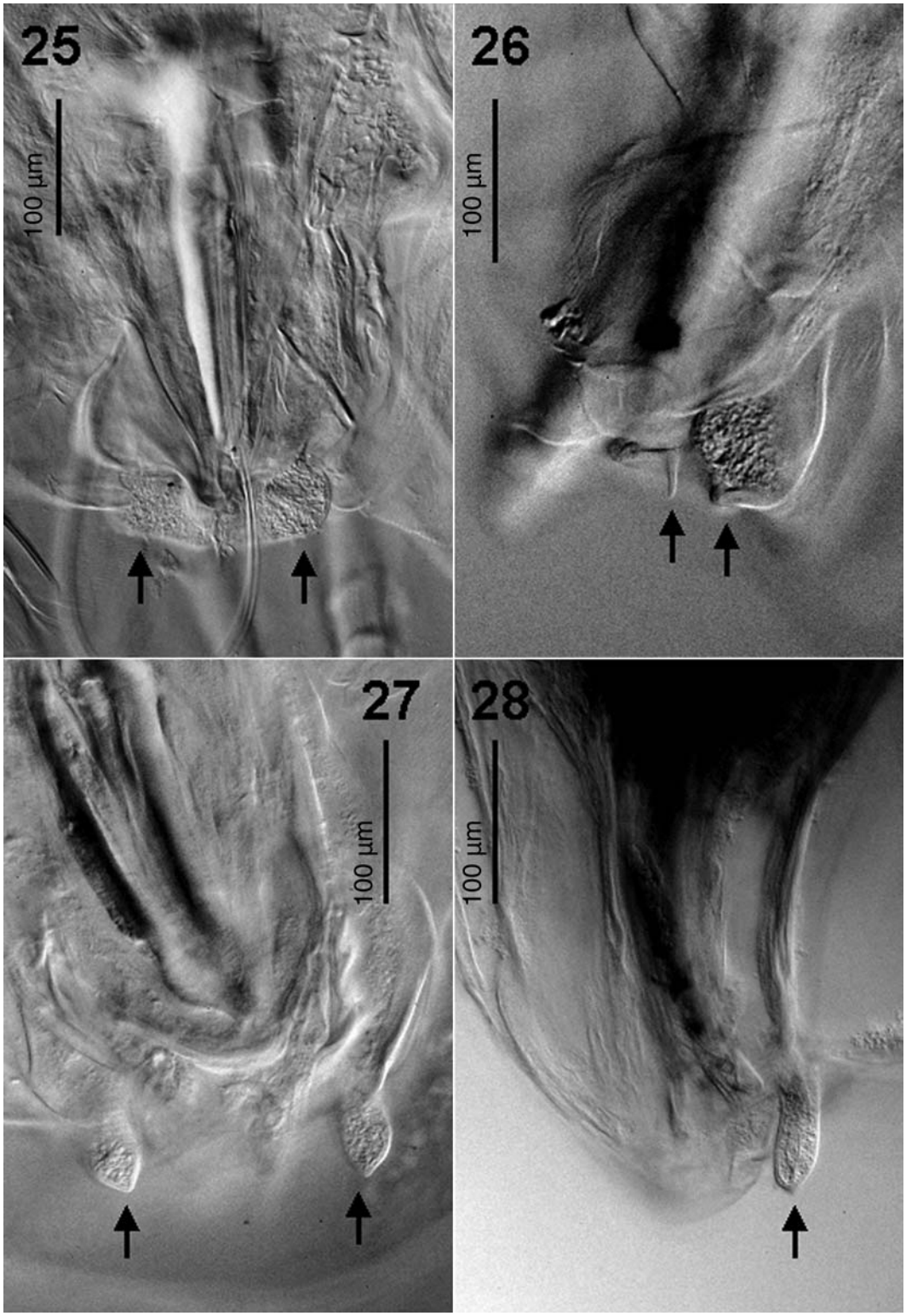
Figures 15–24. Photomicrographs of cephalic characteristics of *Cylicocyclus insigne* and *Cylicocyclus gyalocephaloides*. **15.** *Cylicocyclus insigne*, dorsoventral view, showing buccal capsule, lateral cephalic papillae, and elements of external leaf-crown. **16.** *Cylicocyclus insigne*, right lateral view, showing buccal capsule, low mouth collar, and large esophageal funnel with a thin cuticular lining. **17.** *Cylicocyclus gyalocephaloides*, dorsoventral view, showing buccal capsule, lateral cephalic papillae, and elements of external leaf-crown projecting through the mouth. **18.** *Cylicocyclus gyalocephaloides*, lateral view, showing buccal capsule, low mouth collar, and large esophageal funnel with a thin cuticular lining. **19.** *Cylicocyclus insigne*, lateral view, showing elements of internal leaf-crown (left arrow) at top edge of buccal capsule and support (right arrow) for elements of external leaf-crown. **20.** *Cylicocyclus insigne*, showing submedian papilla. **21.** *Cylicocyclus insigne*, lateral view, showing short, thin, rod-like elements of internal leaf-crown (arrow). **22.** *Cylicocyclus gyalocephaloides*, lateral view, showing elements of internal leaf-crown (left arrow) at top edge of buccal capsule and support (right arrow) for elements of external leaf-crown. **23.** *Cylicocyclus gyalocephaloides*, showing submedian papilla. **24.** *Cylicocyclus gyalocephaloides*, lateral view, showing short, thin, rod-like elements of internal leaf-crown (arrow).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and differentiating in horses. A phylogeny of *Cylicocyclus* spp. is needed.

The question of whether the older name, *C. zebrae*, should replace *C. gyalocephaloides* was considered. Because no types of *C. zebrae* were found, we propose that it remain a forgotten name. Types of *C. gyalocephaloides* are available, and this name has been used in all recent literature for this species. In the interest of stability, the overriding principle of the International Code of Zoological Nomenclature, *C. gyalocephaloides* should continue as the name of this cyathostomin of *E. burchelli*.

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Figures 25–28. Photomicrographs of genital cones of male *Cylicocyclus insigne* and *Cylicocyclus gyalocephaloides*. **25.** *Cylicocyclus insigne*, ventral view, showing broad, paired dorsal papillae (arrows) of genital cone. **26.** *Cylicocyclus insigne*, left lateral view, showing one of paired dorsal papillae (right arrow) and cuticular spine (left arrow) ventral to the dorsal papilla. **27.** *Cylicocyclus gyalocephaloides*, ventral view, showing slender, paired dorsal papillae (arrows) of genital cone. **28.** *Cylicocyclus gyalocephaloides*, left lateral view, showing 1 of paired dorsal papillae (arrow).